View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Lirias

Immunotherapy in Uterine Cancers

137

An Coosemans, MD, PhD, Sandra Tuyaerts, PhD
Anke Vanderstraeten, Frederic Amant, MD, PhD

Introduction

Uterine cancers can be divided in uterine carcinoma and the rare group of uterine sarcoma. Uterine high grade sarcomas in general and recurrent endometrial carcinomas are highly aggressive. Standard treatment modalities are poor in number and have limited success. There is a clear need for better and less toxic treatment modalities for uterine tumors (1, 2).

During tumor growth, the immune system interacts with and attempts to destroy the tumor. The immune system discriminates malignant cells from their benign counterparts because tumor cells aberrantly express tumor-associated antigens (TAA). The observation that tumors are frequently infiltrated by tumor infiltrating lymphocytes (TIL) led to the development of the cancer immunosurveillance theory by Burnet and Thomas in 1957. In this view, tumor growth can be regarded as a multistep process termed "immunoediting" during which the immune system continuously interacts with and attempts to destroy the tumor. The appearance of clinically detectable tumors can thus be explained as a failure of the immune system to control the tumor (3). This is probably related to the link between inflammation and cancer, implicating that the chronic activation of the immune system ultimately leads to immune dysfunction. Supporting this is the fact that several chronic inflammatory diseases are associated with an increased risk of cancer (e.g. inflammatory bowel disease and colon cancer, prostatitis and prostate cancer, hepatitis and liver cancer) (4). Although several immune cells can have an anti-tumor role (e.g. mature DC, cytotoxic T lymphocytes, NK cells), the chronic inflammatory tumor microenvironment inhibits their function, thus contributing to immune escape.

Immunotherapy can be defined as the treatment of cancer by inducing, enhancing or suppressing an immune response. Immunotherapy is based on the recognition of TAA, leading to the induction of a specific anti-tumor response without harming healthy surrounding tissue. There are two distinct types of immunotherapy, namely active and passive immunotherapy. Active immunotherapy implies cancer vaccines, where the immune response has to be elicited by the human body. This can be achieved in several ways: injection of a (modified) peptide/protein, vaccination with recombinant DNA or viral vectors, cellular therapy using gene-modified tumor cells or tumor antigen loaded dendritic cells (DC). The differences and advantage of DC immunotherapy over the other techniques is that DC are professional antigen presenting cells (APC) capable of inducing naïve anti-tumor T cells, whereas the other modalities require uptake by host cells, which include DC but also non-professional APC (5). Passive immunotherapy implies the administration of preformed specific immunotherapeutic agents, tumor-specific antibodies or T cells. The administration of monoclonal antibodies against tumor antigens in HER2-positive breast cancer (Trastuzumab), CD20-expressing B-cell lymphomas (Rituximab), and head and neck, lung, and colorectal cancers that express the epidermal growth factor receptor (Cetuximab) are the most known and clinically effective representatives of this group (6). However, it is also possible to administer tumor-specific T cells, which are either expanded from TIL or genetically modified to express a known antigen-specific T cell receptor (TCR) (7). Experiences in this field are limited and mainly focused in melanoma. Passive immunotherapy can also focus on the modulation of immunosuppressive effects (CTLA-4, PD-1, IDO,…), though clinical trials are still limited with remarkable side-effects for some agents (8, 9).

However, tumor cells change rapidly and are thereby able to escape immune surveillance, resulting in immunological tolerance of the body to cancer. The challenge of immunotherapy is to break this tolerance and to tilt the effect towards immune surveillance.

The Immune System in Uterine Tumors

Endometrial carcinoma displays several features of inflammation, including cytokine secretion and leukocyte infiltration in tumors. It is hypothesized that hormonal alterations and genetic changes (e.g. NF-κB activation, COX-2 upregulation,…) in tumor cells create a pro-inflammatory milieu that facilitates cancer progression (4). Several cytokines and chemokines have been associated with endometrial carcinoma, among which TNFα, IL-6, IL-8, TGF-β. Another soluble mediator indicating chronic immune activation is neopterin, which is increased in endometrial carcinoma (10). Moreover, neopterin production is associated with indoleamine 2,3-dioxygenase (IDO) activity, a mechanism implicated in the suppression of T cell responses which is involved in endometrial cancer (11). Endometrial cancer cells also have been shown to express molecules to escape anti-tumor immunity, e.g. B7-H4, COX-2, CXCR4, STAT3.

In addition to mediators expressed by tumor cells themselves, both innate and adaptive immune cells are recruited into endometrial tumors. Tumor-associated macrophages (TAMs) have pleiotropic functions, which can influence

tumor growth. The role of TAMs in endometrial carcinoma is complex. Located in close contact with cancer cells, they have a beneficial effect on relapse-free survival, but TAMs located in necrotic tumor tissue are linked with tumor recurrence (12). Moreover, TAMs secrete matrix metalloproteinases and induce angiogenesis which promote tumor progression (13). Neutrophils are also increased in endometrial cancer, but their exact role is still enigmatic. Endometrial tumors are frequently infiltrated by T lymphocytes as well. Tumor-specific $CD4^+$ and $CD8^+$ T cells are implicated in tumor eradication. Hence, the presence of $CD8^+$ cytotoxic T lymphocytes at the invasive border of endometrioid endometrial carcinoma leads to a more favorable outcome for the patient (14), suggesting an effective cytotoxic effect of these cells. On the other hand, $CD8^+$ lymphocytes present in primary untreated stage IA-IIIA endometrial cancer, show upregulation of inhibitory natural killer receptors, an effect that possibly is mediated through TGF-β. However, this upregulation prevents the cytotoxic function of these lymphocytes at the HLA recognition level, thus causing an important tumor evasion strategy, since non-classical HLA molecules are upregulated in cancer (15). $CD4^+$ FoxP3-expressing regulatory T cells (Treg) play an important role in the prevention of auto-immunity and in the controlled downregulation of undesired immune responses. Unfortunately, in many tumors, they will also suppress endogenous and induced antitumor immune responses. However, in endometrial carcinoma, the presence of Treg is debatable. Fattorossi *et al.* showed their presence in the tumor-draining lymph nodes of 26 patients (16), whereas Giatromanolaki *et al.* demonstrated less Treg in 79 stage I endometrial carcinoma patients compared to normal endometrium (17). More recently, the field of myeloid-derived suppressor (MDSC) cells has gained more attention. They have multiple action pathways, including inhibition of the innate and the adaptive immunity, supporting tumor angiogenesis, facilitation of Treg development and limiting the availability of mature and functional DCs (18). To our knowledge, the role of these cells has not been explored in endometrial carcinoma.

The role of the immune system in uterine sarcoma is much less evident and poorly studied. However, a recent publication showed the association of uterine sarcoma with systemic inflammation, as measured by an enhanced neutrophil to lymphocyte ratio in these patients (19). TIL can be detected in uterine sarcoma, but these cells can sometimes harbor chromosomal aberrations which might negatively impact their functionality (20). Like in endometrial carcinomas, neopterin levels have been shown to be increased in patients with uterine sarcoma, indicating the activation of cell-mediated immunity (21).

Immunotherapy in Uterine Tumors

Although ample research has been done in several malignant disorders, such as prostate cancer, malignant glioma and melanoma, little has been done to date in uterine tumors, in spite of, albeit quite limited, evidence supporting uterine tumors as a valid target for immunotherapy. Several tumor antigens have for example been described for these tumors yet remained largely unexplored in an immunotherapeutic setting. In addition, several groups have reported immune system involvement in uterine tumors, described in the previous section. The immunotherapeutic strategies that have been explored so far in uterine cancer are further elaborated on in the following sections, after which possible new strategies arising from other cancer types or *in vitro* observations will be discussed.

Immunotherapy For Endometrial Carcinomas

Table 1 gives an overview of immunotherapeutic strategies that have been tested *in vitro*, in animals or in humans. Three major conclusions can be drawn.

First, there are only a few reports in the *in vitro* or animal setting, and of those strategies that have been explored first at experimental level, only one was brought to clinic. The opposite is true as well: most clinical attempts in endometrial carcinoma have no fundamental basis. It is true that the majority of the techniques has been explored in other tumors, both fundamentally and clinically, but *in vitro* or animal work with endometrial carcinoma cells preceding most clinical attempts in Table 1 are non existing. At first, this seems surprising. At second, it seems more or less acceptable. Immunotherapy cannot be seen separately from the existing immune mechanisms in the tumor and the human body, which are undoubtedly influencing the clinical result of the therapy. This effect can be partially mimicked in mice but is not fully representative for the *in vivo* situation in humans.

Secondly, immunotherapy in endometrial carcinoma is in its infancy. The studies are very limited in number and grouped in time. The oldest immunotherapeutic attempt dates already from 1987 (22) but between 1991 and 1999, the interest for immunotherapy in endometrial carcinoma was lost. Eventually, only 13 studies have been published, using very diverse techniques. Four of them use adoptive T cell transfer (22-25), four studies – the most recent ones - focus on a tumor-associated antigen (WT1, EpCAM, TF) (26-29), two studies use a general anti-tumor agent (tumor necrosis factor alpha and gonadotropin-releasing hormone analogue conjugates (30, 31)), two studies use DC immunotherapy (27, 32) and three studies are published using the injection of a peptide, of which there was direct (26, 33) or indirect (34) evidence that the peptide/protein was also present in the tumor. This diversity makes it difficult to draw firm conclusions about the effectiveness of a specific therapy or to select one to explore further.

Immunotherapy For Uterine Sarcomas

Table 2 gives an overview of the immunotherapy trials in uterine sarcoma. The situation is even worse compared to the endometrial carcinoma group. Only 3 clinical studies, less than a decade old, with a total of 4 patients have been published (26,33,35). There are no *in vitro* or animal data available. Although this is disappointing for an aggressive disease, it is not surprising. Uterine sarcomas are rare and

Table 1. Immunotherapeutic strategies tested in vitro.

Author	N	Type of carcinoma	Phase	Procedure	Results
Inoue M *et al*, 1987 (22)	1	Endometrial carcinoma	Animal study	Adoptive transfer of lymphokine-activated killer (LAK) cells	Severe growth retardation of the tumor in nude mice
Shimizu H *et al*, 1989 (23)	3	Endometrial carcinoma	Animal study	Adoptive transfer of lymphokine-activated killer (LAK) cells +/- rIL-2 +/- lentinan in nude mice inoculated with endometrial carcinoma cell lines	Tumor growth is inhibited
Steis RG *et al*, 1990 (24)	1	Endometrial carcinoma	Clinical phase I	Adoptive transfer of lymphokine-activated killer (LAK) cells + rIL-2 intraperitoneally	PD
Hersh EM *et al*, 1991 (30)	2	Endometrial carcinoma	Clinical phase II	Injection of recombinant tumor necrosis factor alpha	Not specified
Palyi I *et al*, 1999 (31)	1	Endometrial adenocarcinoma	In vitro	Application of gonadotropin-releasing hormone analogue conjugates on cell lines	Strong decrease of colony formation and proliferation of tumor cells
Santin A *et al*, 2000 (25)	1	Endometrioid endometrial carcinoma	Case report	Injection of specific T cells, created by the in vitro challenging of T cells with DCs loaded with whole tumor lysate	Transient decrease of tumor marker, stabilization of tumor marker during therapy, infiltration of these specific T cells into small tumoral lesions
Santin A *et al*, 2002 (32)	3	Serous endometrial carcinoma	Clinical phase I	Injection of DCs loaded with whole tumor lysate	All patients induced CD8+ CTL, able to kill autologous tumor cells in vitro
Tsuda N *et al*, 2004 (33)	1	Endometrial carcinoma	Clinical phase I	Injection of maximal 4 peptides to which specific T cells were detectable in blood + Montanide ISA51	PD after 2m*
Kaumaya PT *et al*, 2009 (34)	2	Endometrial carcinoma	Clinical phase I	Injection of two chimeric, B-cell epitopes derived from HER2 extracellular domain in a combination vaccine with a promiscuous T-cell epitope (ie, MVF) and normuramyl-dipeptide as adjuvant emulsified in SEPPIC ISA 720	1 PR for 4 years and 1 no clinical response
Ohno S *et al*, 2009 (26)	1	Endometrioid endometrial carcinoma	Clinical phase I	Injection of a modified 9-mer WT1 peptide	PD after 3m*
Coosemans A *et al*, 2010 (27)	1	Serous endometrial carcinoma	Case report	Injection of WT1-RNA loaded DC	Transient decrease of tumor marker and immunological response
Cocco E *et al*, 2010 (group Santin) (28)	3	Serous endometrial carcinoma	In vitro	Chromium release assay after application of human immuno-conjugate molecule (hI-con1) (= antibody-like molecule targeted against tissue factor (TF)) on cell lines	65.6% of tumor cells was killed
El-Sahwi *et al*, 2010 (group Santin) (29)	5	Serous endometrial carcinoma	In vitro	Chromium release assay after application of MT201 (adecatumumab), a human monoclonal antibody against EpCAM, on cell lines	33% of tumor cells was killed

*counted from the start of immunotherapy

therefore studies are hard to set up. *In vitro* or animal work could be set up with commercial cell lines, though the most used commercial cell lines SK-UT-1 and MES-SA are old lines. Since the knowledge about the histology of uterine sarcomas has changed tremendously over time, the exact diagnosis of SK-UT-1 and MES-SA then is questionable now and thus not completely reliable for further experiments. The culture of new primary tumors would be ideal for further experiments, though this technique is hampered by its risk of overgrowth by fibroblasts.

Search For New Strategies

New Targets: Tumor Associated Antigens

Immunotherapeutic targets can consist of one or more defined TAA or a tumor-derived mixture of unknown TAA. Several TAA are validated for immunotherapy of uterine tumors such as for example several cancer testis antigens (36), MUC1 (37), universal TAA such as hTERT or antigens targeting tumor cell survival such as survivin. Despite their description in uterine tumors, as well as encouraging animal studies using the same antigens in different pathological conditions or *in vivo*

Table 2. Immunotherapy trials in uterine sarcoma.

Author	Number	Type of sarcoma	Phase	Procedure	Results
Hernando JJ *et al*, 2002 (35)	2	Uterine sarcoma	Clinical phase I	Injection of DCs loaded with whole tumor lysate and KLH (Keyhole Limpet Hemocyanin)	One patient had PD after 3 m, the other patient after 6m*
Tsuda N *et al*, 2004 (33)	1	Carcinosarcoma	Clinical phase I	Injection of maximal 4 peptides to which specific T cells were detectable in blood + Montanide ISA51	PD after 5m*
Ohno S *et al*, 2009 (26)	1	Carcinosarcoma	Clinical phase I	WT1 peptide immunotherapy	PD after 3m*

*counted from the start of immunotherapy

human studies in other gynecological tumors (38) or non-gynecological tumors, many of these antigens have remained largely unexplored in an immunotherapeutic setting.

Until now the majority of antigen-focused immunotherapeutic studies in general have used a single antigen as a target. However, more and more arguments are arising for settings using a combination of multiple antigens. There are three major advantages to this type of approach. First of all, by using a combination of several antigens, the risk of immune escape is reduced. Secondly, by using multiple antigens, the group of tumors and hence the patient group that can be targeted with one treatment approach is much larger. For example, patients suffering from tumors that are negative for one particular antigen, but positive for another may benefit from the same therapy if both those antigens are present as a target in the treatment cocktail. Lastly, by including anti-apoptosis factors as a target, the survival of the tumor itself can be attacked as well.

Finally, as an alternative to selected antigens, an immunotherapeutic strategy using all of the antigens present in the tumor (e.g. using total tumor RNA) can be used. This treatment, however, calls for a certain amount of caution, due to the possible occurrence of autoimmune responses, even though a certain amount of autoimmunity is necessary for efficient tumor eradication. Nevertheless, it should be closely monitored.

Immunomodulators

As described above, antibodies targeting inhibitory immune checkpoints have recently emerged as potent anti-cancer drugs that release the brakes of the immune system. The most prominent example is anti-CTLA-4 (Ipilimumab or Tremelimumab) that acts by countering the negative feedback that is induced upon T cell activation in order to limit the immune response. Ipilimumab has been shown to have a beneficial effect on overall survival in phase III trials in melanoma and has also been explored in prostate cancer, pancreatic cancer, non-Hodgkin lymphoma, renal cell cancer, albeit to lesser extent. However, the use of Ipilimumab is associated with severe immune-related adverse events that often correlate with clinical efficacy; these side effects can usually be easily managed by steroid treatment, without affecting the anti-tumor immune response (8,39). Another immune checkpoint that has been explored as a target in cancer therapy is PD-1. PD-1 is an inhibitory receptor expressed on activated T cells and its ligands, PD-L1/B7-H1 and PD-L2/B7-DC, are frequently expressed by tumor cells. Recently, the anti-PD-1 antibody MDX-1106 was tested in phase I clinical trial and reported to be safe and well tolerated, with some anti-tumor activity. More importantly, anti-PD-1 antibody treatment seems to be associated with less immune-related adverse events compared to Ipilimumab (9).

Other tumor-induced immunosuppressive mechanisms activated in uterine tumors (IDO, COX-2, STAT3, Treg) can also be targeted by specific agents. *In vitro* treatment of endometrial cancer cells with curcumin has been shown to result in decreased IL-6 production and decreased IL-6 induced STAT3 phosphorylation and thus constitutes a promising new anti-cancer drug (40). In another *in vitro* study, the COX-2 inhibitor NS-398 was able to inhibit endometrial cancer cell proliferation, viability and invasion (41).

Immunomodulatory drugs (IMiDs) are thalidomide analogues which possess immunomodulatory, anti-angiogenic, anti-inflammatory and anti-proliferative effects. Till now, IMiDs have been mostly tested in hematological malignancies, but they could possibly also be beneficial in solid tumors (42). It remains to be elucidated which of the *in vitro* properties of IMiDs will mediate their prominent mode of action *in vivo*. The parent drug thalidomide has been tested in uterine cancer, but showed limited or no efficacy, so it remains to be elucidated whether the optimized analogues will show enhanced effectiveness (43, 44).

Adoptive T Cell Ttherapy

Adoptive T cell therapy is based on using cancer patient immune cells that are grown outside of the patients body and re-infused in much larger numbers (7). This kind of therapy can be combined with other treatments such as chemotherapy or the addition of IL-2 in order to increase its efficacy. Most data so far concerning adoptive T cell transfer have been obtained in melanoma, by the group of Rosenberg, the first reports dating back to 1988 (*Rosenberg et al* in (7)). Melanoma was chosen as a target because of frequent observations of TIL and the presence of many described TAA. This type of approach has also already been tested in uterine tumors, as discussed above (22-25), with mostly encouraging outcome.

Using adoptive T cell transfer, several important factors hampering the antitumor T cell response can be circumvented by *in vitro* manipulation, reviewed by Hawkins et al. (7). Due to the *in vitro* culturing of T cells, immune regulation causing reduced *in vivo* expansion of antitumor T cells can be

circumvented. In addition, T cell properties can be genetically modified *in vitro*. This technique is often used to generate so-called chimeric antigen receptors (CAR), combining the antigen specificity of an antibody and the killing capacity of a T cell and thereby improving the antitumor capacity of adoptively transferred T cells (45). However, several concerns have arisen in studies using this type of immunotherapy, such as significant "on-target toxicity" (*Morgan et al* in (7)), and toxicity due to mispairing of endogenously present and genetically introduced TCR (*Schumacher et al*. in (7)). The occurrence of these side effects added to the fact that transduced T cells have the capacity to sustain themselves for a very long period of time, which may cause an accumulation of adverse events (45), warrants for careful design of clinical trial using adoptive T cell transfer as an immunotherapeutic strategy.

Cancer Vaccines

Cancer vaccines seek to induce a tumor specific immune response, distinct from self antigens and to provide long term memory to prevent tumor recurrence. Cancer vaccines can be designed in several ways.

First, antigens can be loaded onto APC, most often DC, leading to a T cell response. Moreover, DCs have also been shown to be strong activators of natural killer cells and natural killer T cells, thus linking innate and adaptive immune responses. DC can be cultured *in vitro* starting from monocytes (46) and they can be loaded with one or more TAA or with whole tumor cell products (obtained by tumor lysates, total tumor RNA,…) or with a combination of defined TAA and whole tumor products, which is a prerequisite for tumors without well defined TAA, such as glioblastoma (47). DC immunotherapy has been applied in several tumors with varying success, of which melanoma and glioblastoma are the biggest representatives (48, 49).

Secondly, TAA can be incorporated into a viral vaccine vector (amongst them poxvirus-based and adenovirus-based vectors) which is injected in APC or taken up by APC after cell death of the initially vector-infected cell, mostly epithelial cells. This is a less explored strategy, though it has been used in ovarian cancer and prostate cancer (50). The advantages are their ease to be engineered and their capacity to carry large amounts of genetic material. The disadvantage is that the antibody response to the viral proteins dominates over the desired response of the encoded TAA.

Thirdly, a cancer vaccine can consist solely of antigen-derived peptide(s) that elicit an immune response. Although promising and largely explored, its dependence of the MHC antigen status of the patient and the fact that not all MHC-restricted peptides of a tumor specific protein are yet known, are two serious limitations.

Fourthly, TAA can be delivered through DNA vaccines. DNA vaccines are safe and can easily be manufactured. Because of their low immunogenicity level, DNA fusion vaccines have been developed. TAA can be associated with pro-inflammatory molecules like toll-like receptor agonists to activate APC or they can be fused CD4 epitopes like the fragment C of tetanus toxin, critical for the induction of long-term antitumor immunity (51).

Combination of Immunotherapy With Existing Treatments

It has become clear that the success of immunotherapy is not only the merit of the injected product, but the whole of existing immunotolerance and immunosurveillance in the human body. Moreover, immunotherapy by itself might result in the rise of inhibitory cells (52). Therefore, it is broadly accepted that, in order to be effective, immunotherapeutic agents should be combined with other modalities. In this section, promising combinations of immunotherapeutic agents with other treatment modalities will be highlighted; however, since few studies are performed in uterine cancer, concepts from other tumors will be discussed.

Combination With Chemotherapy

The combination of chemotherapy and immunotherapy may have beneficial results in this respect. Some examples of chemotherapy suppressing inhibitory cells have already been explored in literature. Doxorubicin and paclitaxel at maximally tolerated dose were shown to help break tolerance to "self" cancer antigens and enhanced cancer vaccine efficacy. The exact molecular function of these drugs on particular immune cells subsets has recently started to be clarified. Also, gemcitabine used at conventional doses was found to significantly reduce the number of MDSCs in the spleen of treated mice, whereas other leukocytes were not affected. Finally, lower doses of chemotherapy have been proven synergistic with immunotherapy by depleting Treg and retaining memory T cells in mice, as exemplified for cyclophosphamide (53).

Besides inhibiting immunosuppressive cells, the beneficial effects of chemotherapy could be attributed to the induction of immunogenic tumor cell death, which increases the antigen uptake by antigen presenting cells, leading to *in situ* immunization against tumor antigens. This is the case for doxorubicin. Chemotherapy might also lead to direct activation of mature DC (this was proven for doxorubicin and paclitaxel) or their effector mechanisms. In this respect, the chemotherapeutic dose is critical, since too high doses can negatively impact immune cell function.

The combination with chemotherapy can result in striking effects. As an example, we highlight the study of Antonia *et al* (54). Twenty-nine patients with extensive stage small cell lung cancer were treated with p53-DC immunotherapy. Although half of the population had an immunological response, clinical response was absent in 96.5%. However, if patients received chemotherapy after DC treatment, 62% showed a clinical response, thus improving overall survival, compared to the historic response rate in this group. The clinical response was closely associated with the presence of an immunological response.

Another mechanism that could attribute to the synergy between chemotherapy and immunotherapy is the fact that some mechanisms of chemotherapeutic drug resistance are associated with expression of proteins that are targets of T cell responses. This is exemplified by the induction of survivin upon cisplatin resistance in ovarian cancer (55). Thus, immunotherapy targeting these antigens is appealing

to combine with chemotherapy (56). In addition, explaining the success of the combination of chemotherapy with immunotherapy may be due to the fact that a chemotherapy response exposes new/other antigenic epitopes, to which an efficient immune response can subsequently be arisen.

Combination With Targeted Therapies

The combination of immunotherapy with standard therapy might even result in a synergistic effect. To this respect, especially tyrosine kinase inhibitors which were originally developed as anti-angiogenic drugs show great potential because of their immunomodulatory effects on different immune cell types. Specifically for WT1, a case study combining Imatinib and WT1 peptide immunotherapy in a chronic myeloid leukemia patient has been described. The residual disease that was left from the treatment with Imatinib alone was reduced if both treatments were combined (57). Another tyrosine kinase inhibitor, Sunitinib, also shows great potential for combination with immunotherapeutic agents, because of its beneficial effects on type 1 immune responses and inhibitory effects on both Treg and MDSC (58, 59). The combination of Sipuleucel-T (APC vaccine loaded with prostatic acid phosphatase) with Bevacizumab (anti-VEGF) has been explored in prostate cancer patients resulting in PSA declines and alterations in PSA doubling time (60).

Combination of Different Immunotherapeutic Regimens

Several cytokines have been described to increase the potential of immunotherapeutic regimens. Some are predicted to function through APC (e.g. IFNα, IL-12 and GM-CSF), while other cytokines selectively support activation or maintenance of the effector T cell repertoire (e.g. IL-2, IL-7, IL-15, IL-18, IL-21). However, dosing needs to be performed cautiously, since some cytokines can have considerable side effects (e.g. IFNα, IL-2) or could induce inhibitory immune cells (e.g. IL-2, GM-CSF) (61). Immune activators like Toll-like receptor agonists that induce *in vivo* cytokine secretion could mediate the same effects. However, their use is confounded by their limited availability in GMP quality and their toxicity profile upon systemic administration. Nonetheless, encouraging data have been reported with regard to the combination of different vaccine modalities with TLR agonists like imiquimod, CpG oligonucleotides and monophosphoryl lipid A (MPL) (62).

The combination of an anti-tumor vaccine with blockade of immune inhibitory mechanisms like CTLA-4 or PD-1 is an attractive combination. However, studies combining anti-CTLA4 antibodies Ipilimumab or Tremelimumab with a gp100 or MART1 vaccine in melanoma patients emphasizes that careful timing of both treatments is probably critical (8, 63). PD-1 blockade combined with a GM-CSF secreting tumor vaccine has been shown to induce improved anti-tumor responses in a mouse model (64).

Removal of inhibitory cell populations such as Treg and MDSC is also a promising option for combination with a vaccine regimen. However, effective drugs to deplete these cell types are still under investigation and conflicting observations have been made in cancer patients. For example, low-dose cyclophosphamide, anti-CD25 (Daclizumab), IL-2 immunotoxin (denileukin diftitox, ONTAK) all have been described to significantly deplete Treg which could not always be reproduced by others. This is probably due to the lack of a good Treg marker in humans, which makes it difficult to compare studies where different markers were used. Nevertheless, promising results have been reported using Treg depleting regimens and tumor vaccines (65, 66). Selective depletion of MDSC has not been reported to date. However, several drugs have been reported to mediate inhibition of MDSC maturation, accumulation, function or differentiation, such as sunitinib, celecoxib, all-trans retinoic acid (ATRA), aminobisphosphonates, nitroaspirin and others (59,67). Importantly, the therapeutic effects of many of these compounds are only clear when used in combination with immunotherapy, emphasing that combination of immunotherapy with MDSC inhibition is, at present, a very promising anti-tumor strategy (67).

Finally, the combination of adoptive T cell therapy with cancer vaccines has shown promising results in early clinical trials in cancer patients. However, this kind of therapy is associated with significant hurdles concerning cost and technical challenges (68).

Evaluation Criteria

As a consequence of the complex biological interactions induced by immunotherapy without or with chemotherapy, there is a need to develop new response evaluation criteria for cancer vaccine therapy. The commonly used RECIST criteria (Response Evaluation Criteria in Solid Tumours) (69) are based on cytotoxic therapies that are directed to all dividing cells i.e. chemotherapy. This may lead to dramatic responses, even for bulky disease. Despite this, responses may be shortlasting. Immunotherapy is not expected to induce a speedy response with significant tumor reduction. The added value of immunotherapy lies in prolonged disease stabilization. This is however not measured in the commonly used RECIST criteria. Moreover, stabilization after initial progression during immunotherapy is probably also a favorable outcome. Overall survival and time to progression seem to be more appropriate to evaluate the effect of immunotherapy. These considerations were recently taken into account to evaluate a population treated with Ipilimumab. The authors declared 4 new response patterns, all associated with a favorable survival : (a) shrinkage in baseline lesions, without new lesions; (b) durable stable disease (in some patients followed by a slow, steady decline in total tumor burden); (c) response after an increase in total tumor burden; and (d) response in the presence of new lesions (70).

Conclusion

Recurrent endometrial carcinoma and high grade uterine sarcoma are highly aggressive and reluctant to current treatment modalities and therefore new treatment options are needed. Worldwide, targeted therapies are gaining importance in the field of cancer treatment, among which immunotherapy. However, in uterine cancer it is a relatively new field. Attempts have been made, though the applied therapies were very diverse and not fundamentally (*in vitro*

and animal research) based, so that firm conclusions about effectiveness cannot be drawn. Nevertheless, increasing evidence becomes available pointing to a role of the immune system in uterine cancer, indicating that immunotherapy holds much promise. Moreover, proofs-of-concept from other malignancies also favors the combination of immunotherapy with existing therapies such as chemotherapy. Therefore, the immunotherapeutic field should deserve more attention in future fundamental and clinical research in uterine cancer.

References

1. Amant F, Coosemans A, Debiec-Rychter M, Timmerman D, Vergote I. Clinical management of uterine sarcomas. Lancet Oncol 2009;10: 1188-98.
2. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet 2005;366: 491-505.
3. Sengupta N, MacFie TS, MacDonald TT, Pennington D, Silver AR. Cancer immunoediting and "spontaneous" tumor regression. Pathol Res Pract 2010;206: 1-8.
4. Wallace AE, Gibson DA, Saunders PT, Jabbour HN. Inflammatory events in endometrial adenocarcinoma. J Endocrinol 2010;206: 141-57.
5. Dougan M, Dranoff G. Immune therapy for cancer. Annu Rev Immunol 2009;27: 83-117.
6. Weiner LM, Surana R, Wang S. Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nat Rev Immunol 2010;10: 317-27.
7. Hawkins RE, Gilham DE, Debets R, et al. Development of adoptive cell therapy for cancer: a clinical perspective. Hum Gene Ther 2010;21: 665-72.
8. Hodi FS, O'Day SJ, McDermott DF, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. N Engl J Med 2010.
9. Brahmer JR, Drake CG, Wollner I, et al. Phase I Study of Single-Agent Anti-Programmed Death-1 (MDX-1106) in Refractory Solid Tumors: Safety, Clinical Activity, Pharmacodynamics, and Immunologic Correlates. J Clin Oncol 2010.
10. Melichar B, Solichova D, Freedman RS. Neopterin as an indicator of immune activation and prognosis in patients with gynecological malignancies. Int J Gynecol Cancer 2006;16: 240-52.
11. Ino K, Yamamoto E, Shibata K, et al. Inverse correlation between tumoral indoleamine 2,3-dioxygenase expression and tumor-infiltrating lymphocytes in endometrial cancer: its association with disease progression and survival. Clin Cancer Res 2008;14: 2310-7.
12. Ohno S, Ohno Y, Suzuki N, et al. Correlation of histological localization of tumor-associated macrophages with clinicopathological features in endometrial cancer. Anticancer Res 2004;24: 3335-42.
13. Iurlaro M, Loverro G, Vacca A, et al. Angiogenesis extent and expression of matrix metalloproteinase-2 and -9 correlate with upgrading and myometrial invasion in endometrial carcinoma. Eur J Clin Invest 1999;29: 793-801.
14. Kondratiev S, Sabo E, Yakirevich E, Lavie O, Resnick MB. Intratumoral CD8+ T lymphocytes as a prognostic factor of survival in endometrial carcinoma. Clin Cancer Res 2004;10: 4450-6.
15. Chang WC, Huang SC, Torng PL, et al. Expression of inhibitory natural killer receptors on tumor-infiltrating CD8+ T lymphocyte lineage in human endometrial carcinoma. Int J Gynecol Cancer 2005;15: 1073-80.
16. Fattorossi A, Battaglia A, Ferrandina G, et al. Lymphocyte composition of tumor draining lymph nodes from cervical and endometrial cancer patients. Gynecol Oncol 2004;92: 106-15.
17. Giatromanolaki A, Bates GJ, Koukourakis MI, et al. The presence of tumor-infiltrating FOXP3+ lymphocytes correlates with intratumoral angiogenesis in endometrial cancer. Gynecol Oncol 2008;110: 216-21.
18. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009;9: 162-74.
19. Kim HS, Han KH, Chung HH, et al. Neutrophil to lymphocyte ratio for preoperative diagnosis of uterine sarcomas: a case-matched comparison. Eur J Surg Oncol 2010;36: 691-8.
20. Engel H, Friedrich J, Kleespies C, et al. Detection of chromosomal aberrations in tumor cells and tumor infiltrating lymphocytes by molecular cytogenetics in patients with gynecological cancer. Cancer Genet Cytogenet 1998;106: 159-65.
21. Fuith LC, Fuchs D, Hausen A, et al. Urinary neopterin excretion in patients with uterine sarcomas. Cancer 1990;65: 1228-31.
22. Inoue M, Shimizu H, Shimizu C, et al. Antitumor efficacy of recombinant interleukin 2-activated killer cells against endometrial cancers. Nippon Sanka Fujinka Gakkai Zasshi 1987;39: 143-4.
23. Shimizu H, Inoue M, Tanizawa O. Adoptive cellular immunotherapy to the endometrial carcinoma cell line xenografts in nude mice. Gynecol Oncol 1989;34: 195-9.
24. Steis RG, Urba WJ, VanderMolen LA, et al. Intraperitoneal lymphokine-activated killer-cell and interleukin-2 therapy for malignancies limited to the peritoneal cavity. J Clin Oncol 1990;8: 1618-29.
25. Santin AD, Hermonat PL, Ravaggi A, et al. Development and therapeutic effect of adoptively transferred T cells primed by tumor lysate-pulsed autologous dendritic cells in a patient with metastatic endometrial cancer. Gynecol Obstet Invest 2000;49: 194-203.
26. Ohno S, Kyo S, Myojo S, et al. Wilms' tumor 1 (WT1) peptide immunotherapy for gynecological malignancy. Anticancer Res 2009;29: 4779-84.
27. Coosemans A, Wolfl M, Berneman ZN, et al. Immunological response after therapeutic vaccination with WT1 mRNA-loaded dendritic cells in end-stage endometrial carcinoma. Anticancer Res 2010;30: 3709-14.
28. Cocco E, Hu Z, Richter CE, et al. hI-con1, a factor VII-IgGFc chimeric protein targeting tissue factor for immunotherapy of uterine serous papillary carcinoma. Br J Cancer 2010;103: 812-9.
29. El-Sahwi K, Bellone S, Cocco E, et al. Overexpression of EpCAM in uterine serous papillary carcinoma: implications for EpCAM-specific immunotherapy with human monoclonal antibody adecatumumab (MT201). Mol Cancer Ther 2010;9: 57-66.
30. Hersh EM, Metch BS, Muggia FM, et al. Phase II studies of recombinant human tumor necrosis factor alpha in patients with malignant disease: a summary of the Southwest Oncology Group experience. J Immunother (1991) 1991;10: 426-31.
31. Palyi I, Vincze B, Lovas S, et al. Gonadotropin-releasing hormone analogue conjugates with strong selective antitumor activity. Proc Natl Acad Sci U S A 1999;96: 2361-6.
32. Santin AD, Bellone S, Ravaggi A, et al. Induction of tumour-specific CD8(+) cytotoxic T lymphocytes by tumour lysate-pulsed autologous dendritic cells in patients with uterine serous papillary cancer. Br J Cancer 2002;86: 151-7.
33. Tsuda N, Mochizuki K, Harada M, et al. Vaccination with predesignated or evidence-based peptides for patients with recurrent gynecologic cancers. J Immunother 2004;27: 60-72.
34. Kaumaya PT, Foy KC, Garrett J, et al. Phase I active immunotherapy with combination of two chimeric, human epidermal growth factor receptor 2, B-cell epitopes fused to a

promiscuous T-cell epitope in patients with metastatic and/or recurrent solid tumors. J Clin Oncol 2009;27: 5270-7.
35. Hernando JJ, Park TW, Kubler K, Offergeld R, Schlebusch H, Bauknecht T. Vaccination with autologous tumour antigen-pulsed dendritic cells in advanced gynaecological malignancies: clinical and immunological evaluation of a phase I trial. Cancer Immunol Immunother 2002;51: 45-52.
36. Brooks N, Pouniotis DS. Immunomodulation in endometrial cancer. Int J Gynecol Cancer 2009;19: 734-40.
37. Sivridis E, Giatromanolaki A, Koukourakis MI, Georgiou L, Anastasiadis P. Patterns of episialin/MUC1 expression in endometrial carcinomas and prognostic relevance. Histopathology 2002;40: 92-100.
38. Dobrzanski MJ, Rewers-Felkins KA, Quinlin IS, et al. Autologous MUC1-specific Th1 effector cell immunotherapy induces differential levels of systemic TReg cell subpopulations that result in increased ovarian cancer patient survival. Clin Immunol 2009;133: 333-52.
39. Weber J. Ipilimumab: controversies in its development, utility and autoimmune adverse events. Cancer Immunol Immunother 2009;58: 823-30.
40. Saydmohammed M, Joseph D, Syed V. Curcumin suppresses constitutive activation of STAT-3 by up-regulating protein inhibitor of activated STAT-3 (PIAS-3) in ovarian and endometrial cancer cells. J Cell Biochem 2010;110: 447-56.
41. Yi Z, Jingting C, Yu Z. Proteomics reveals protein profile changes in cyclooxygenase-2 inhibitor-treated endometrial cancer cells. Int J Gynecol Cancer 2009;19: 326-33.
42. Knight R. IMiDs: a novel class of immunomodulators. Semin Oncol 2005;32: S24-30.
43. McMeekin DS, Sill MW, Benbrook D, et al. A phase II trial of thalidomide in patients with refractory endometrial cancer and correlation with angiogenesis biomarkers: a Gynecologic Oncology Group study. Gynecol Oncol 2007;105: 508-16.
44. McMeekin DS, Sill MW, Darcy KM, et al. A phase II trial of thalidomide in patients with refractory leiomyosarcoma of the uterus and correlation with biomarkers of angiogenesis: a gynecologic oncology group study. Gynecol Oncol 2007;106: 596-603.
45. Brenner MK, Heslop HE. Adoptive T cell therapy of cancer. Curr Opin Immunol 2010;22: 251-7.
46. Tuyaerts S, Aerts JL, Corthals J, et al. Current approaches in dendritic cell generation and future implications for cancer immunotherapy. Cancer Immunol Immunother 2007;56: 1513-37.
47. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol 2005;5: 296-306.
48. Ardon H, Van Gool S, Lopes IS, et al. Integration of autologous dendritic cell-based immunotherapy in the primary treatment for patients with newly diagnosed glioblastoma multiforme: a pilot study. J Neurooncol 2010.
49. Jacobs JF, Punt CJ, Lesterhuis WJ, et al. Dendritic cell vaccination in combination with anti-CD25 monoclonal antibody treatment: a phase I/II study in metastatic melanoma patients. Clin Cancer Res 2010;16: 5067-78.
50. Gulley JL, Arlen PM, Tsang KY, et al. Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. Clin Cancer Res 2008;14: 3060-9.
51. Tsen SW, Paik AH, Hung CF, Wu TC. Enhancing DNA vaccine potency by modifying the properties of antigen-presenting cells. Expert Rev Vaccines 2007;6: 227-39.
52. Lehe C, Ghebeh H, Al-Sulaiman A, et al. The Wilms' tumor antigen is a novel target for human CD4+ regulatory T cells: implications for immunotherapy. Cancer Res 2008;68: 6350-9.
53. Kandalaft LE, Singh N, Liao JB, et al. The emergence of immunomodulation: combinatorial immunochemotherapy opportunities for the next decade. Gynecol Oncol 2010;116: 222-33.
54. Antonia SJ, Mirza N, Fricke I, et al. Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. Clin Cancer Res 2006;12: 878-87.
55. Chock KL, Allison JM, Shimizu Y, ElShamy WM. BRCA1-IRIS overexpression promotes cisplatin resistance in ovarian cancer cells. Cancer Res 2010;70: 8782-91.
56. Andersen MH, Junker N, Ellebaek E, Svane IM, Thor Straten P. Therapeutic cancer vaccines in combination with conventional therapy. J Biomed Biotechnol 2010;2010: 237623.
57. Oji Y, Oka Y, Nishida S, et al. WT1 peptide vaccine induces reduction in minimal residual disease in an Imatinib-treated CML patient. Eur J Haematol 2010;85: 358-60.
58. Finke JH, Rini B, Ireland J, et al. Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients. Clin Cancer Res 2008;14: 6674-82.
59. Ko JS, Zea AH, Rini BI, et al. Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. Clin Cancer Res 2009;15: 2148-57.
60. Rini BI, Weinberg V, Fong L, Conry S, Hershberg RM, Small EJ. Combination immunotherapy with prostatic acid phosphatase pulsed antigen-presenting cells (provenge) plus bevacizumab in patients with serologic progression of prostate cancer after definitive local therapy. Cancer 2006;107: 67-74.
61. Ferrantini M, Capone I, Belardelli F. Dendritic cells and cytokines in immune rejection of cancer. Cytokine Growth Factor Rev 2008;19: 93-107.
62. Steinhagen F, Kinjo T, Bode C, Klinman DM. TLR-based immune adjuvants. Vaccine 2010.
63. Ribas A, Comin-Anduix B, Chmielowski B, et al. Dendritic cell vaccination combined with CTLA4 blockade in patients with metastatic melanoma. Clin Cancer Res 2009;15: 6267-76.
64. Li B, VanRoey M, Wang C, Chen TH, Korman A, Jooss K. Anti-programmed death-1 synergizes with granulocyte macrophage colony-stimulating factor--secreting tumor cell immunotherapy providing therapeutic benefit to mice with established tumors. Clin Cancer Res 2009;15: 1623-34.
65. de Vries IJ, Castelli C, Huygens C, et al. Frequency of Circulating Tregs with Demethylated FOXP3 Intron 1 in Melanoma Patients Receiving Tumor Vaccines and Potentially Treg-Depleting Agents. Clin Cancer Res 2011;17: 841-8.
66. Rech AJ, Vonderheide RH. Clinical use of anti-CD25 antibody daclizumab to enhance immune responses to tumor antigen vaccination by targeting regulatory T cells. Ann N Y Acad Sci 2009;1174: 99-106.
67. Ugel S, Delpozzo F, Desantis G, et al. Therapeutic targeting of myeloid-derived suppressor cells. Curr Opin Pharmacol 2009;9: 470-81.
68. Rapoport AP, Aqui NA, Stadtmauer EA, et al. Combination immunotherapy using adoptive T-cell transfer and tumor antigen vaccination on the basis of hTERT and survivin after ASCT for myeloma. Blood 2011;117: 788-97.
69. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92: 205-16.
70. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15: 7412-20.